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Form and Distribution of Neurons in Rat Cingulate Cortex: Areas 32, 24, and 29

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ABSTRACT The cytoarchitecture of rat cingulate cortex is described. This includes the topographical distribution and layering patterns of Brodmann's areas 25, 32, 24, and 29a, b, c, and d. Area 24 is subdivided into a ventral area 24a and a dorsal area 24b, but an area 23 could not be identified between areas 24 and 29.

An analysis of Golgi impregnations in areas 32, 24, and 29 demonstrates that most neuronal types recognized in neocortical areas are also present in cingulate cortex. Besides typical and inverted pyramidal cells, there is a wide variety of nonpyramidal cells, including multipolar, bitufted, and bipolar cells. Small multipolar cells with small somata, a dendritic tree limited to one or two layers, sparse to moderately spinous dendrites and one of two varieties of short axonal trajectories are present in layers I and II of areas 32, 24, and 29d. Medium multipolar cells occur mainly in layers III and V; they have extensive dendritic trees which traverse three or more layers, moderately spinous dendrites, and an axonal plexus which either ascends or descends in the cortex. Large multipolar cells are also frequent in layers III and V; their extensive dendritic trees are essentially spine free and they have axons which form dense terminations, particularly in the layer above the one in which the cell body is located.

Neurons with elongated somata and a primarily vertical orientation of the dendritic tree are either bitufted or bipolar. Bitufted cells are most frequent in layers II and III of areas 32, 24, and 29d. These cells have dendritic trees which form "hourglass shaped" fields, dendrites which are moderately spinous, and axons which form either extensive horizontal and vertical projections or are "chandelier" in form. Bipolar cells, in contrast, are found in layers II–V; their sparsely spinous dendrites form narrow dendritic trees which are oriented vertically and extend across four or more layers, and their axons have the same vertical orientation as the dendritic tree.

It is concluded that the form of the axonal arbors of nonpyramidal cells frequently mimics the extent and shape of their dendritic trees. Thus, small multipolar cells with limited, spherical dendritic trees may have axons which arch sharply and emit short, terminal branches. In contrast, medium and large multipolar cells have more extensive dendritic and axonal arbors which traverse two, three, or more layers. Of the fusiform cells, bitufted ones with their "hourglass" dendritic trees have extensive vertical and horizontally oriented axonal branches, while bipolar cells have narrow, vertically oriented dendritic and axonal arbors.

The granular layers II–IV of area 29c contain the following types of neurons: small and fusiform pyramids, medium-sized pyramids, large stellate cells, and medium multipolar cells. Fusiform pyramids are the only neurons unique to cingulate cortex. They are similar to the variety of pyramidal cells, but have an oval soma and only one basal dendrite which extends from the base of the cell body to arborize in layer IV. Large stellate cells differ from large multipolar cells in that they have densely spinous dendrites and axons which enter the white matter.

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The medial cortical surface of the rat brain receives numerous pharmacologically unique brainstem afferents, including ones which are serotonergic, dopaminergic, and noradrenergic (Palkovits et al., '77; Azmitia and Segal, '78; Lidov et al., '80; Lindvall et al., '74; Emson and Koob, '78; Lewis et al., '79; Swanson and Hartman, '75; Jones et al., '77). A number of other studies suggest that the individual neurons in cingulate cortex may bear unique receptor complexes. Thus, Hunt and Schmidt ('78) have found that some cells in layer II might actually endocytose the αbungarotoxin ligand which binds specifically to acetylcholine receptors, while Sar et al. ('78) have identified enkephalinergic processes around neurons which they suggest might be pyramidal cells. However, despite these studies of afferent connections and receptor binding in the cingulate cortex of the rat, there is no clear account of the morphologically distinct areas that exist in this cortex, and additionally, very little is known about the form and distribution of neurons.

With respect of the cytoarchitecture of the rat cingulate cortex, a number of inconsistencies have developed in the literature. For example, Kreig's ('46a,b) account of the parcellation of the medial cortex of the rat, the account upon which most experimental anatomical studies rely, describes only a limited distribution for Brodmann's ('09) area 32, and it fails to show two subdivions of area 29. Furthermore, although Kreig ('46a,b) recognized an area 23 between areas 24 and 29, as did Brodmann ('09), Caviness ('75) and Krettek and Price ('77) do not describe area 23 as part of the cingulate cortex of either the mouse or rat.

The present study will first evaluate cingulate cortex cytoarchitecture in the rat in the light of Brodmann's ('09) original description. Second, a systematic classification of neurons based upon their dendritic and axonal trajectories will be presented on the basis of surveys of Golgi impregnations of areas 32, 24, and 29, to extend the accounts published by Cajal ('11, '22).

MATERIALS AND METHODS

Cytoarchitecture

A 200-gm, 46-day-old, hooded (Long-Evans) male rat was anesthetized with 0.5 ml of 36% chloral hydrate and perfused intracardially with 0.9% sodium chloride followed by 10% formalin. The brain was postfixed in 10% formalin for 2 weeks and then dehydrated and embedded in low-viscosity nitrocellulose (Tissue Embedding Solution, Randolph Products Carlstadt, New Jersey). Six series of coronal sections were prepared; the first four were 50μm thick, while the remaining two were 25-μm thick. A standard cresyl violet procedure was then followed to stain two series, one of each thickness.

A number of other male, hooded (Long-Evans) and albino (Sprague-Dawley) rats were also perfused and fixed as described above, but instead of celloidin embedding, the brains were surrounded with an albumin-gelatin mixture and 25μm coronal sections were cut on a freezing microtome. In addition to cresyl violet staining, another series of sections was prepared for studying normal axon distribution according to a reduced silver protocol. This procedure involves leaving the sections overnight in a solution of silver nitrate (2.5%): pyridine, in a ratio of 8:1, and then reducing the silver with formaldehyde in the series of solutions used in the Fink-Heimer procedure for silver staining of degenerated terminals (Vogt, '74).

Golgi preparations

Twenty-four hooded or albino male rats ranging from 15 days to 6 months of age were anesthetized with 36% chloral hydrate (0.1 ml/100 gm body weight) and artifically respired with 95% O_2/5% CO_2. The thorax was opened, 0.5 ml of 1% sodium nitrite injected into the heart, and then 100 ml of a solution containing 1% paraformaldehyde, 1.25% glutaraldehyde, and 0.015% calcium chloride in a 0.08 M cacodylate buffer at pH 7.2 and warmed to 30–40° C was perfused through the heart. This was followed with 100 ml of a more concentrated solution containg 2% paraformaldehyde and 2% glutaraldehyde in a 0.08 M cacodylate buffer (see Peters, '70, for further details). The heads of these perfused animals were left in the refrigerator overnight and then the brains were removed and stored in the concentrated fixative.

One of two rapid Golgi procedures was used on these brains. For impregnating a limited number of clearly separated neurons the Valverde ('70) procedure was followed. After washing the fixed blocks of cortex for 15 minutes in 0.15 M cacodylate buffer, they were transferred to a solution containing 2.4% potassium dichromate and 0.2% osmium tetrox-

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ide in distilled water (at least 20 ml of solution for each block). Four days later the blocks were washed three times in 0.75% silver nitrate and left in 0.75% silver nitrate for 2 days in the dark. The tissue blocks were then dehydrated, embedded in low-viscosity nitrocellulose, and immediately cut into 125-μm-thick coronal sections which were cleared in terpineol and mounted from xylene.

A second procedure was often used which resulted in impregnating greater numbers of neurons. Although a wider spectrum of cells could be studied in this material, an intermingling of processes, particularly distal axons, often limited the use of this type of preparation. In this procedure (Valverde, '65) the tissue blocks were left in concentrated fixative in the refrigerator for at least 7 days, after which they were placed into a solution of 1.92% potassium dichromate and 0.56% osmium tetroxide in the dark for 7 days. The blocks were then washed and left in silver nitrate before being processed in the same way as those impregnated by the first procedure. Impregnated neurons were drawn with a Zeiss microscope and camera lucida attachment at a magnification of 425 × and in some instances drawn again at approximately 1250 ×.

RESULTS

Cytoarchitecture

A map of the topographical distribution of areas found on the medial surface of the rat cerebral hemisphere is presented in Figure 1. Anterior divisions are quite similar in extent, although not in nomenclature, to those recognized by Krettek and Price ('77), while posterior divisions of area 29 more closely resemble those defined by Brodmann ('09). Although a cingulate gyrus with delimiting callosal and cingulate sulci, as in primates, is not present per se in the rat, cingulate cortex in the rat can be defined as that portion of the cortex on the medial surface of the hemisphere which surrounds the callosal sulcus. Within this portion of cortex areas 25, 32, 24, and 29 can be distinguished.

The six cytoarchitecturally distinct layers now generally recognized to exist in neocortical areas are not all present in most cingulate cortical areas in the rat. Thus, anterior areas 25, 32, and 24 lack a layer of small cells that can be equated with layer IV of the neocortex, while divisions of area 29 have a very dense outer band of neurons which can not be separated into layers II and III.

Area 25 lies dorsal to the tenia tecta and has three cellular layers: a dense external layer II–III whose border with layer I is irregular, an inner layer V which has slightly larger pyramidal cells than those in layer II–III, and a layer VI of somewhat smaller neurons. In Figure 3 the arrow delineates layer II–III from layer V in area 25. Area 32 (Figs. 1, 2, and 3) lies dorsal to area 25, abuts area 24a posteriorally, and has a distinguishable layer II. As in area 25, the neurons in all layers of area 32 are still very densely packed. Layer III of area 32 has cells which are smaller than those in layer V, and the somewhat horizontally elongated neurons in layer VI are sometimes packed into thin sublayers.

Although Brodmann's ('09) area 24 is consistently described in cytoarchitectural studies of the medial surface of the mouse (Rose, '12; Caviness, '75), rat (Kreig, '46a,b; Krettek and Price, '77), and monkey (Walker, '40), Walker recognized very early that this area is composed of a number of cytoarchitectural zones, of which the simplest adjoin the corpus callosum and the more complex occur at the lip of the cingulate sulcus. Smith ('45) made a similar observation, but further parcellation of area 24 was not made until just recently when it was shown that two subareas can be distinguished in area 24. Thus, area 24a (Figs. 2 and 3), which is equivalent to Krettek and Price's ('77) ventral division of the anterior cingulate area, has neurons which are very evenly distributed throughout its entire depth. Area 32 shares this even distribution of neurons, but in area 32 the neurons, particularly those in layer V, are much larger and rounder than in area 24a. A thin layer of large cells comprising layer V is present in area 24a, although it is difficult to distinguish these neurons at low magnifications (Fig. 2), and the same is true of layer II. However, the cell size differences that mark these layers are quite apparent at higher magnification (Fig. 3). In contrast to area 24a, area 24b, which is

Abbreviations

CB Cingulum bundle
CC Corpus callosum
CCr Rostrum of the corpus callosum
CCs Splenium of the corpus callosum
H Hippocampus
OC Optic chiasm
Ps Postsubiculum
tt Tenia tecta

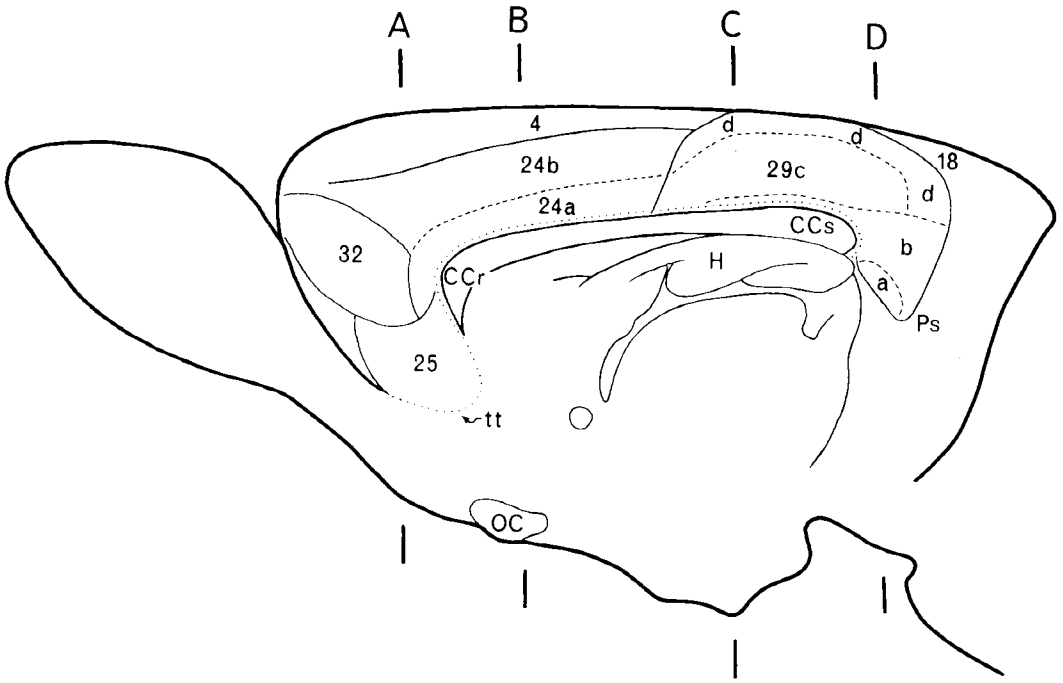

Fig. 1. Topographic distribution of cytoarchitectural areas of cingulate cortex in the rat. A, B, C, and D represent the approximate levels from which sections in Figure 2 were photographed.

equivalent to Krettek and Price's ('77) dorsal division of the anterior cingulate area, has a sparse layer III, so that layer II is clearly visible and there is a broader layer V whose cells are larger than those of area 24a (Fig. 2). Layer VI has neurons that are somewhat smaller than those found in the same layer in area 32.

Another possible location for area 23 in the rat is dorsal to retrosplenial cortex. Rose and Woolsey ('48) demonstrated that their cingular area in the rabbit is dorsal to retrosplenial cortex and has a broad layer IV; they suggested it is equivalent to Brodmann's ('09) areas 29c and 29d (see below). In contrast, primates have clearly separable areas 29d (retrosplenial agranular cortex) and 23, with layer IV of area 23 containing a substantially higher number of stellate cells than area 29d the small size of this strip and its lack of a distinct cytoarchitecture leads us to discount the presence of an area 23 between areas 24 and 29 in the rat.

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Brodmann ('09) observed five subdivisions of posterior or retrosplenial cortex; areas 29a, b, c, d, and e. Area 29a is the thinnest, smallest, least differentiated, and most ventral retrosplenial area. It has a homogeneous granular layer, a layer V with large, rounded pyramidal cells and a layer VI of variable thickness (Fig. 3).

Area 29b has a granular layer which is clearly divisible into a thin, dense zone and a broader, sparsely populated zone beneath (Fig. 3). Although this layer appears granular, it

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On page 606, first column, the wrong text was mistakenly placed in the second paragraph. The second paragraph correctly reads:

> Posterior to area 24 some authors have identified either an area 23 (Brodmann, '09; Rose, '12; Krieg, '46a, b) which would be equivalent to the granular area 23 present in higher species (Vogt, '76) or an area 29 (Caviness, '75; Krettek and Price, '77). Rose ('12) states that on the basis of cytoarchitecture alone it is very difficult to identify an area 23, finding that it can only be distinguished in myelin stained sections which show a moderately dense inner plexiform layer. There is indeed a short transitional region about 1/2 mm wide between areas 24 and 29. However, the small size of this strip and its lack of a distant cytoarchitecture leads us to discount the presence of an area 23 between areas 24 and 29 in the rat.

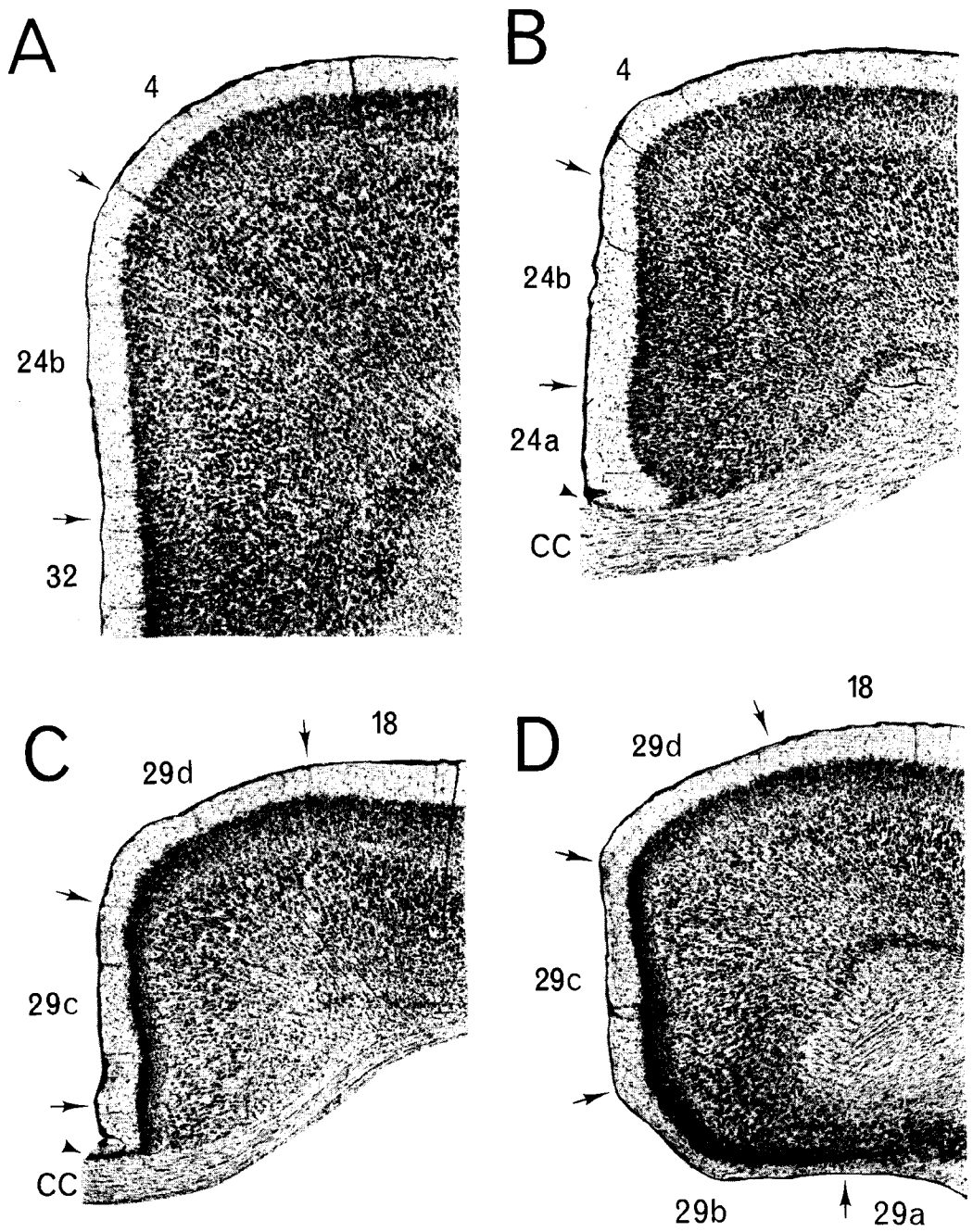

Fig. 2. Four levels of the rat cingulate cortex. Arrowheads in B and C point to the indusium griesium. Cresyl violet stain, 43 ×.

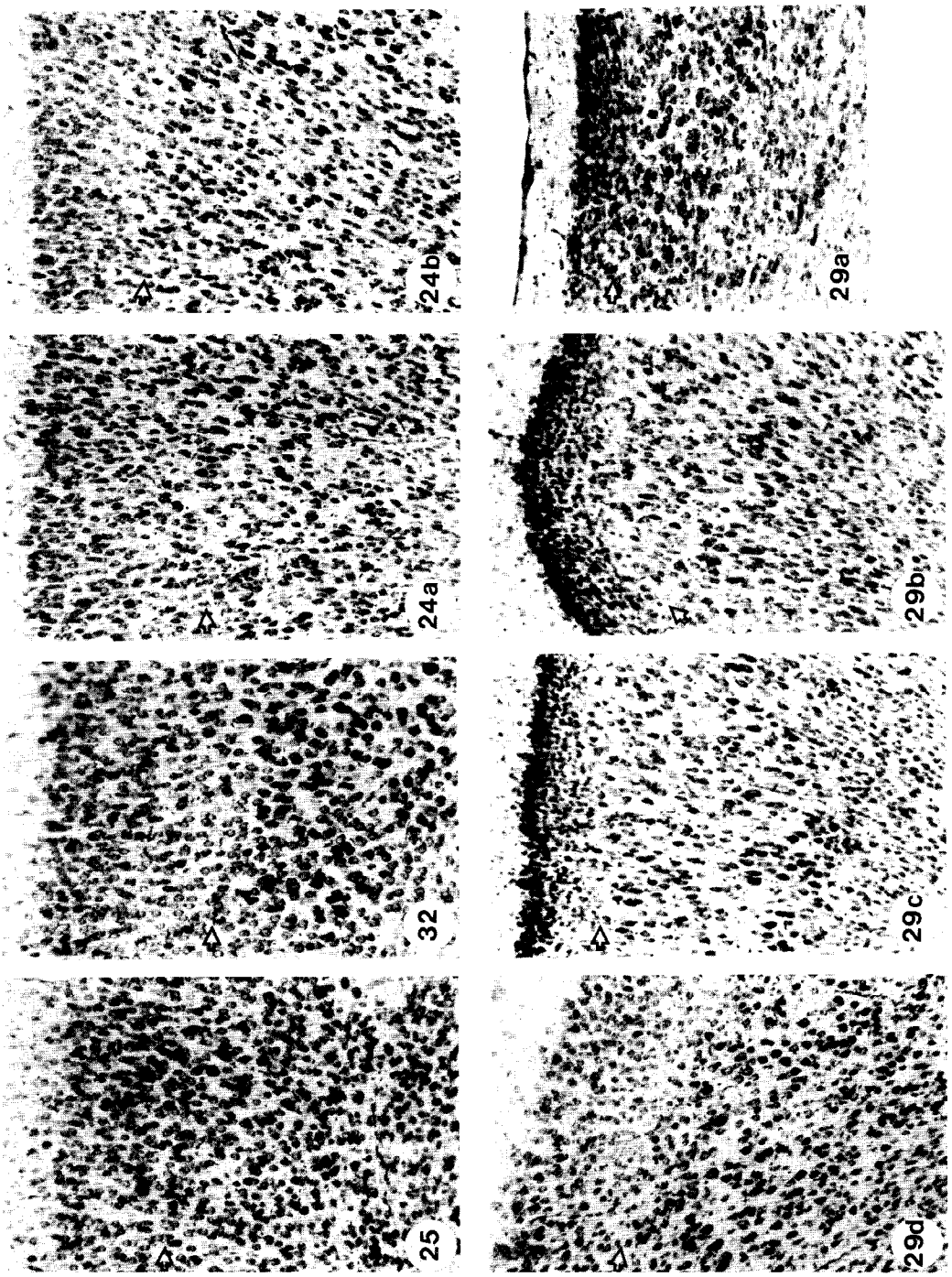

Fig. 3. Cytoarchitectural areas identified in the rat medial cortex. Arrows indicate the border between the superficial layers and layer V. Parts of layers I and VI have been included. Cresyl violet stain, 100 ×.

contains small pyramidal cells and, since the dense granular layer of areas 29b and c is continuous with layers II–III of areas 29d and 18 (Fig. 2, C, D), this layer will be referred to as layer II–III, even though its cellular components differ somewhat from area to area. The underlying sparse granular layer, which also contains a dense, horizontal fiber plexus (Fig. 4), is Brodmann's layer IV. Area 29b continues rostrally above the corpus callosum (Fig. 2, C, D) and has large layer V cells directly abutting layer IV. Area 29c, in contrast, has a thin lamina dessicans (clear zone) between layer IV and that part of layer V which has medium-sized pyramidal cells. Note in Figure 3 that the large pyramids are located quite deep in layer V of area 29c, which tends to have thinner layers II–III and IV than area 29b.

Cajal ('11, '22) recognized seven cortical layers in retrosplenial granular cortex; outer plexiform, large stellate, vertical fusiform, inner plexiform, medium pyramidal, large pyramidal, and polymorph. Cajal's layers of medium and large pyramidal cells are together equivalent to layer V of the now accepted lamination pattern of neocortex. In addition, we have not been able to identify a cytoarchitecturally distinct layer of large stellate cells, since such neurons are intermingled with those of what Cajal considered to be the deeper layer of vertical fusiform cells. Thus, Cajal's second layer of large stellate cells is part of the cell dense composite layer II–III. This interpretation results in the following equivalencies: I = outer plexiform, II–III = large stellate/vertical fusiform, IV = inner plexiform, V = medium and large pyramidal, VI = polymorph.

Retrosplenial agranular cortex of Rose ('12) is equivalent to Brodmann's ('09) area 29d and lies dorsal to area 29c. When compared with area 29c, area 29d has layers II–III which significantly expand in width and have a reduction in cell packing density (Fig. 3). Layer IV has slightly larger and less densely packed neurons and the presence of this layer means that area 29d is not strictly agranular but that it is dysgranular, as is true of this cortex in the monkey (Vogt, '76).

Brodmann ('09) recognized a fifth retrosplenial area 29e posterior and ventral to areas 29b and c. The superficial layers of this area

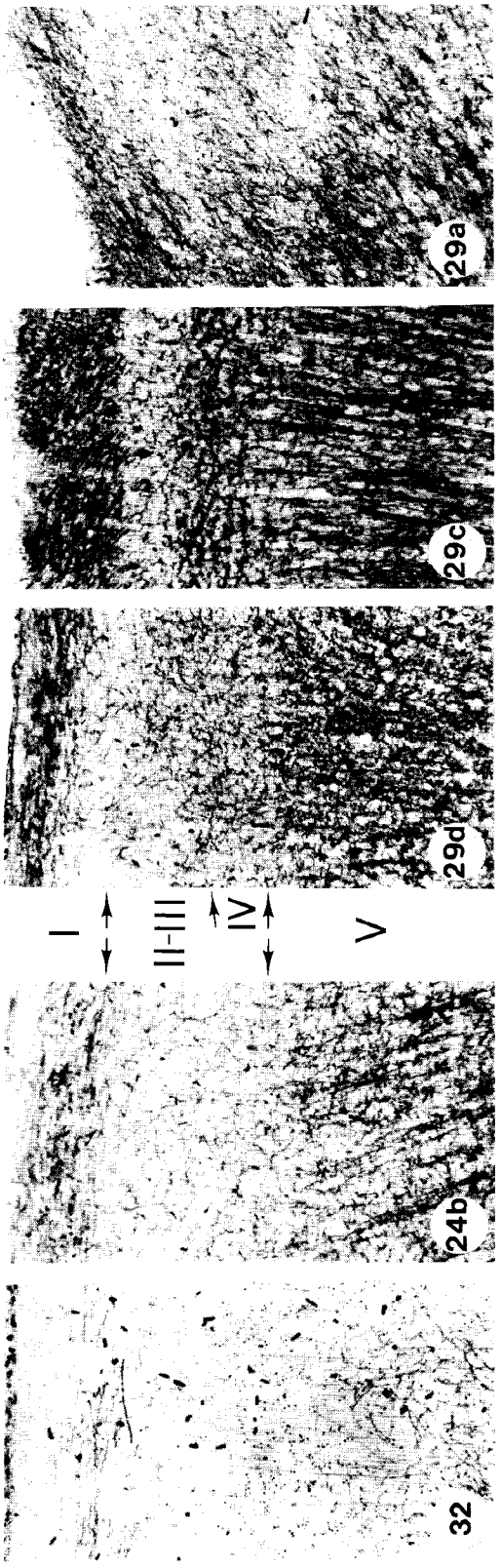

Fig. 4. Distribution of large diameter, normal axons in selected areas of the rat cingulate cortex. Since areas 32 and 24b do not have a layer IV, the arrows indicate the borders between layers I, II–III, and V, while in area 29 they delineate layers I, II–IV, IV, and V. Reduced silver stain, 90 ×.

have an even distribution of neurons that are somewhat larger than in the retrosplenial granular areas, while the pyramidal cells of the deeper layers are smaller than in other areas, resulting in a much more even distribution of neurons throughout this cortex. Since area 29e is unlike the retrosplenial areas, Rose and Woolsey ('48) and Swanson et al. ('78) included it in the postsubicular area—a practice which will be continued in the present study (Fig. 1, Ps).

Besides these cytoarchitectural differences among the cingulate cortical areas, as seen in Figure 4, there are striking differences in the distribution of large diameter, normal axons in many of these areas. The external (layer I) and internal (layer IV) plexiform layers are most pronounced in area 29c, while many vertically oriented axonal bundles are contained in layer V. The lateral portion of area 29a, in contrast, contains a homogeneous distribution of horizontally oriented fibers throughout its entire depth, although more medially the plexiform layers are barely differentiable. In area 29d, where layers II–III are substantially broader than in area 29c, the density of the external plexiform layer is reduced, while the inner plexiform layer is dispersed and no longer has a primarily horizontal orientation.

Anterior cingulate areas have substantially fewer of these large caliber axons. Area 24b has a very poorly differentiated external plexiform layer and no internal plexiform layer, while area 32 does not even have the axonal fascicles so characteristic of layer V in posterior cingulate areas.

Golgi Analysis

Neurons in the cerebral cortex have traditionally been categorized as either pyramidal or stellate (nonpyramidal). Typical pyramidal cells have an orienting apical dendrite which has an apical tuft, the dendrites are moderate to densely spinous, and the descending axon emits three to six collaterals before it enters the white matter. In contrast, stellate or nonpyramidal cells lack these characteristics. Thus, stellate cells do not have an orienting dendrite, tend to have fewer spines per unit length of dendrite and have axons which seem to terminate intracortically without entering the white matter.

Pyramidal cells. Pyramidal cells occur in layers II–VI of all cingulate cortical areas. Three issues of particular interest to pyramidal cell morphology in this cortex will be considered: spine distribution and branching patterns of apical dendrites of layer V and VI cells in area 29c; an analysis of the "vertical fusiform" cell of Cajal ('22) in layers II-IV of areas 29a, b, and c; and inverted pyramidal cell location and morphology.

Layer V pyramidal cells in area 29 have a unique spine distribution and branching pattern when compared to similar cells in neocortex. Neocortical layer V pyramids have an apical dendrite which is free of spines before the first branching site, and more distally the number of spines per unit-length of dendrite increases until a maximal number is reached midway along the apical dendrite (see Marin-Padilla, '67; Schierhorn et al., '72). The density of spines then decreases and in layer I there are few spines per unit-length of dendrite. The apical tufts of neocortical pyramidal cells actually arise throughout layer II and their terminal branches then extend into layer I. In areas 29b and c layer V pyramids have a different distribution of spines, for although the spines attain a maximal concentration at midlevels of the apical dendrite (Fig. 5, n), in layer II–III there is a substantial reduction in spine number and some dendrites are almost completely barren at this point. However, at the very top of layer II–III the apical dendrite branches profusely to enter layer I, and at this site there is another substantial increase in spine density which persists throughout most of layer I. Layer VI pyramidal cells (Fig. 5, o, p), in contrast, generally have thinner dendrites and the apical dendrite spine distribution is like that of neocortical cells.

Fusiform pyramidal cells have small (approximately 8 × 12 μm), fusiform somata within layer II–III of areas 29b and c. Notice in Figure 5 that layer II–III is extremely cell dense and the distribution of the basal dendritic process of these cells (Fig. 5, a, and b) occurs mainly in the deeper, less cell-dense layer IV. Finally, although these cells occur directly beneath layer I, the tapering apical dendrite still forms an apical tuft, in this case in the upper ⅓ to ⅔ of layer I.

The "vertical fusiform" cell of Cajal ('22) has an ovoid or fusiform soma, a bipolar distribution of moderately spinous dendrites, and an axon which emits three or more collaterals before entering the white matter. Since Cajal himself states that this cell has a long cylindrical axon, which is comparable to that of the small pyramid in other cortical regions, it would seem appropriate to reclassify this cell as a fusiform pyramid.

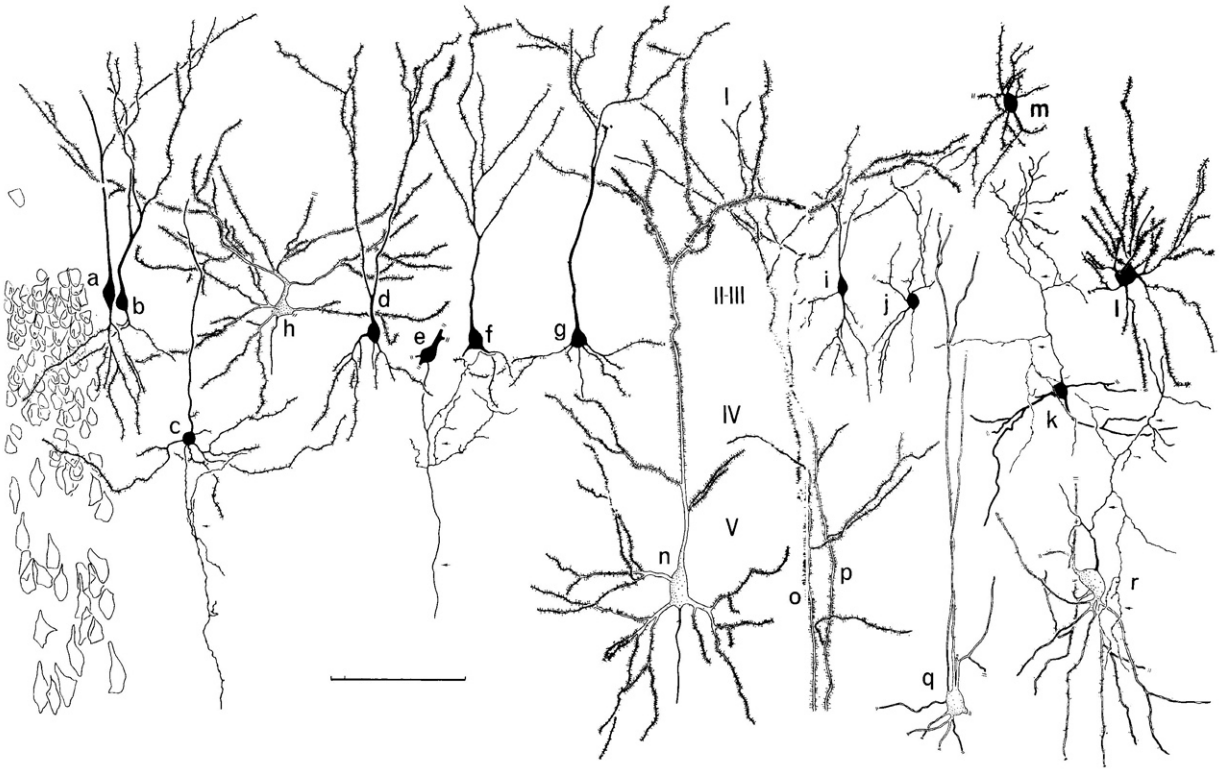

Fig. 5. Cells in layers I–V of area 29c. Cell somata presented on the left are from cresyl violet-stained section. The roman numeral designation for each layer is presented in the middle of this illustration. Fusiform pyramids, a and b; small pyramids, c, d, e (axon collaterals of), f and g; medium pyramid, h; small multipolar, m; medium multipolar, k; large stellate, l; i and j may be small bipolar and multipolar cells respectively; large pyramid with apical branching in layer I, n; apical dendrites of small-medium pyramids of layer VI with branches in layers I and V, o and p; large multipolar cells of layer V with dendritic and axonal processes rising into the granular layer, q and r respectively. Arrows indicate axons, calibration = 100 μm with a 10μm division.

A number of other small pyramidal cells in layers II–III and IV of area 29c have round, pyramidal, or slightly oval somata, but there is a clear skirt of basal dendrites emanating from the base of the cell body (Fig. 5, c, d, e, f, and g). The apical dendrites of these cells have various branching patterns. In one type (cells d and g) primary or secondary branches ascend in layer I and form a tuft which is limited to the upper ⅓ of layer I. This tuft is usually very densely covered by spines and when many tufts are impregnated, they form dense, conical-shaped tangles. In a second variety of apical branching, as exhibited by cell f, the branches are emitted more evenly throughout layer I. The apical branching of small pyramids, therefore, presents either condensed conical-shaped tufts or the apical tuft branches are distributed more evenly throughout all of layer I.

Inverted pyramidal cells share most of the characteristics of typical pyramidal cells except that the orienting, apical dendrite and its terminal tuft are projected toward the white matter (cingulum bundle, Fig. 6) instead of toward the pia. The axons of these cells may originate from the opposite side of the soma, rise, and arch back into the white matter. Alternatively, the axon may emanate from the apex of the soma (Fig. 6, B) and project straight into the cingulum bundle, giving off three to five collaterals within the cortex.

The somata of inverted pyramids are located at the top of layer VI or bottom of layer V. In one unusual Golgi preparation a large number of these cells were impregnated, such that in any one section as many as five to ten cells with overlapping basal dendrites were apparent. This would suggest that a substantial number of inverted pyramids are present in areas 32 and 24b of cingulate cortex.

Multipolar cells. Ideally these cells have dendrites which radiate equally around the soma, and an axon which terminates within

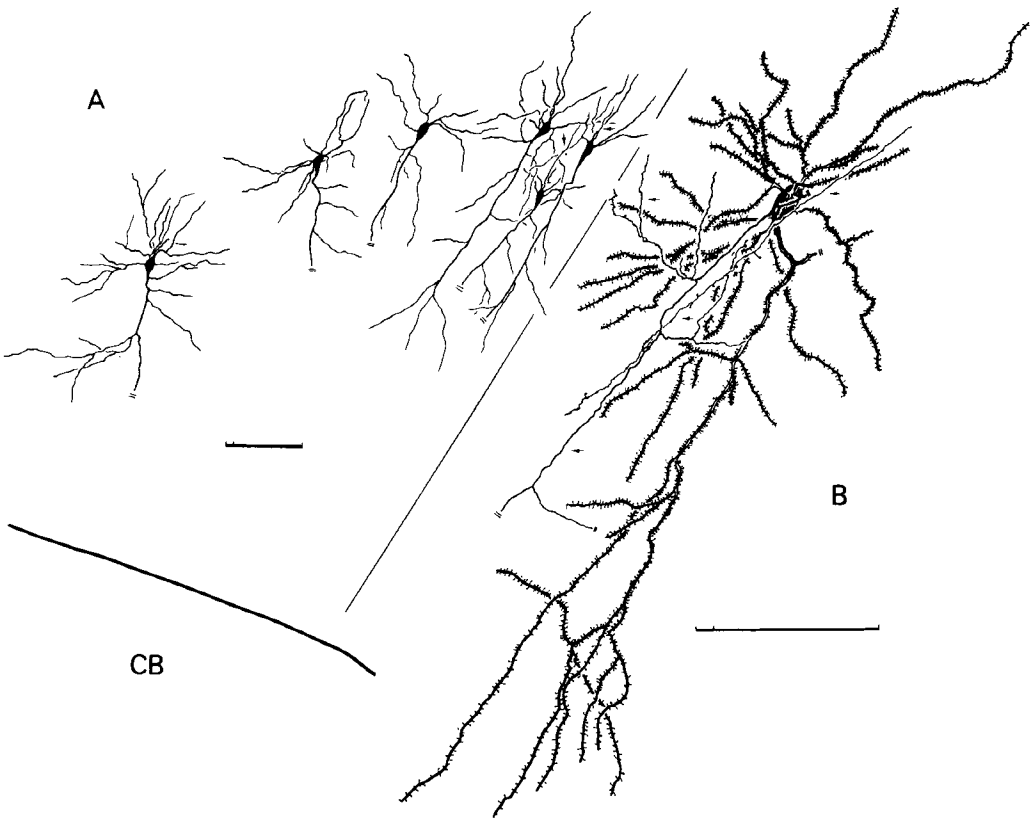

Fig. 6. Inverted pyramidal cells from area 24b just anterior to the rostrum of the corpus callosum. A. Six cells close to the cingulum bundle. B. Higher magnification of a cell from an adjacent section. Arrows indicate axons, Calibration = 100 μm with a 10-μm division.

the cortex. However, cells of this type display a wide variation in somal size, dendritic length, spine density, and axonal distribution patterns, and it is useful to further subdivide this class of cells to account for these variations. Thus, Valverde ('71) described large, medium, and small stellate cells in layer IV of monkey visual cortex and these distinctions can also be useful in considering the multipolar stellate cells of rat cingulate cortex.

Small multipolar cells have small somata (8–12 μm in diameter) and are most frequently found in layers I and II. They possess a relatively confined dendritic tree which is usually limited to one layer (Figs. 7 and 8). Although most of these cells have a moderate number of spines per unit-length of dendrite, the dendrites of some are almost spine free. As with the dendrites, the axons of these cells usually have a short projection field confined to the layer containing the soma or extending into the adjacent layer. Two axonal morphologies can be observed. In the first type the primary axonal stalk arches to form a spherical field and collaterals lacking a particular orientation are emitted at regular intervals (Fig. 7, a, b, c, d, and Fig. 8, a, b). In the second type the axon usually arises from the base of the cell and has short, vertically oriented collaterals with large varicosities (Fig. 8, c, d).

Medium multipolar cells have somata that are 10μm or greater in diameter and are found mainly in layers III and V. The dendritic tree extends long distances to traverse two or more layers (Figs. 9 and 10) and is covered with low to moderate numbers of spines. The initial segment of the axon emanates from either above or below the soma, or from a primary dendrite. The primary axonal stalk then usually projects vertically, giving off six to 12 branches which extend laterally both within the layer of origin and into more superficial or deeper layers. Cell a (Fig. 9), for example, has a soma which is located in layer III of area 24b and an axon that ascends into layer I, while forming terminal branches in layers III and II, and especially in layer I.

Large multipolar cells have somata that are 12–15 μm in diameter and are located mainly in layers III and V, although some are also present in layer II (Figs. 11 and 12). In comparison to the other varieties of multipolar-stellate cells, the dendrites of these neurons are the ones most consistently free of spines. Like those of medium multipolar cells, the dendrites project for long distances, but there is a difference in that more primary dendritic branches extend from the somata of the large multipolar cells.

Although the axons of large multipolar cells ascend or descend in a form similar to those

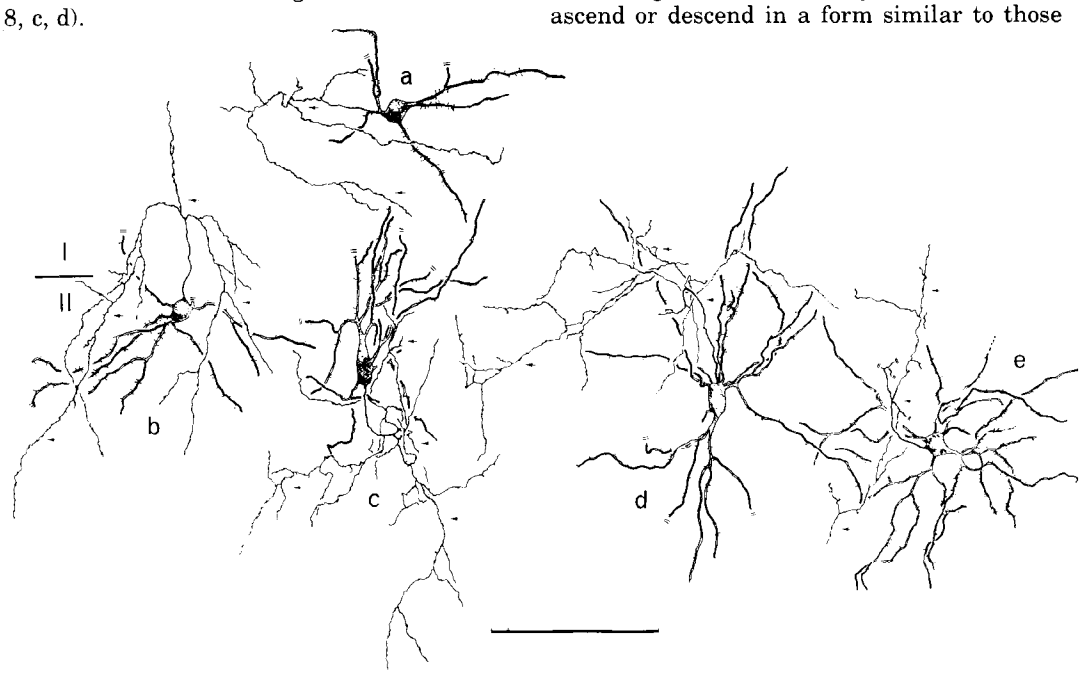

Fig. 7. Small multipolar cells in layers I and II of area 24. Calibration = 100 μm with a 10-μm division.

of the medium multipolar cells, the large cells appear to have a greater number of terminal branches. For example, if cell a in Figure 9, a medium multipolar cell, is compared with cells b and d in Figure 11, which are large multipolar cells, the large cells can be seen to have more terminal axonal branches in layer III than the medium multipolar cell. Furthermore, large multipolar cell a in Figure 12 in layer V has a very extensive arborization in layer II before the primary stalk enters layer II, where it will, no doubt, branch further and extend into layer I.

In conclusion, there are small to large multipolar cells in cingulate cortex with consistent variations in somal, dendritic, and axonal morphology within each subclass. Thus, small multipolar cells are located mainly in the most superficial layers, have limited dendritic trees, and two varieties of short axonal arbors. Medium and large multipolar cells, in contrast, occur mainly in layers III and V and have extensive dendritic and axonal projections. The dendrites of medium-sized cells, though, are moderately spinous and those of large cells spine free, while larger cells have more primary dendritic processes. The axons of large multipolar cells also appear to produce more terminal branches than do those of the medium multipolar cells.

Bitufted cells. Not all nonpyramidal cells in cingulate cortex have round somata and approximately evenly radiating dendrites. The early accounts by Cajal ('11, '22) and Lorente de Nó ('22, '33) of cingulate and other cortical areas describe a general class of fusiform cells which have oval-shaped somata and dendritic trees. Today this class of cells is recognized to include both bitufted and bipolar cells (Feldman and Peters. '78).

Bitufted cells have oval somata approximately 10-μm wide and are most frequent in layers II and III, although they also appear in layers V and VI (Figs. 13, 14, and 15). The dendritic tree of such a cell forms an hourglass with either conical prolongations at each end of the soma from which the secondary dendrites project (Fig. 15, a, b) or tufts of three or more dendrites projecting directly from the top and bottom of the soma (Fig. 14, b). The dendrites of most of these cells are moderately spinous.

Bitufted cells have two varieties of axonal arbors. In one form the primary axon stalk ascends into layer I where it arches back to descend into layer III (Fig. 14). Throughout the initial course in layer III, and possibly in layer II, numerous long (up to 500 μm), horizontal branches are given off. At the arch in layer I, many more collaterals are emitted,

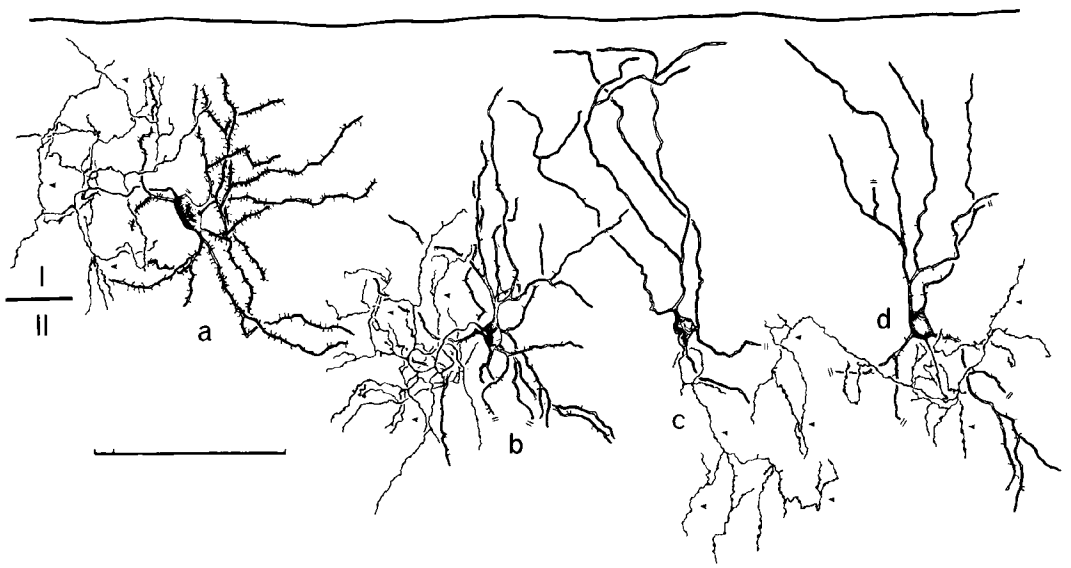

Fig. 8. Small multipolar cells in layers I and II of area 29d. Two variations in axonal morphology (cells a and b versus c and d) can be seen as well as differences in spine density. Calibration = 100 μm with a 10-μm division.

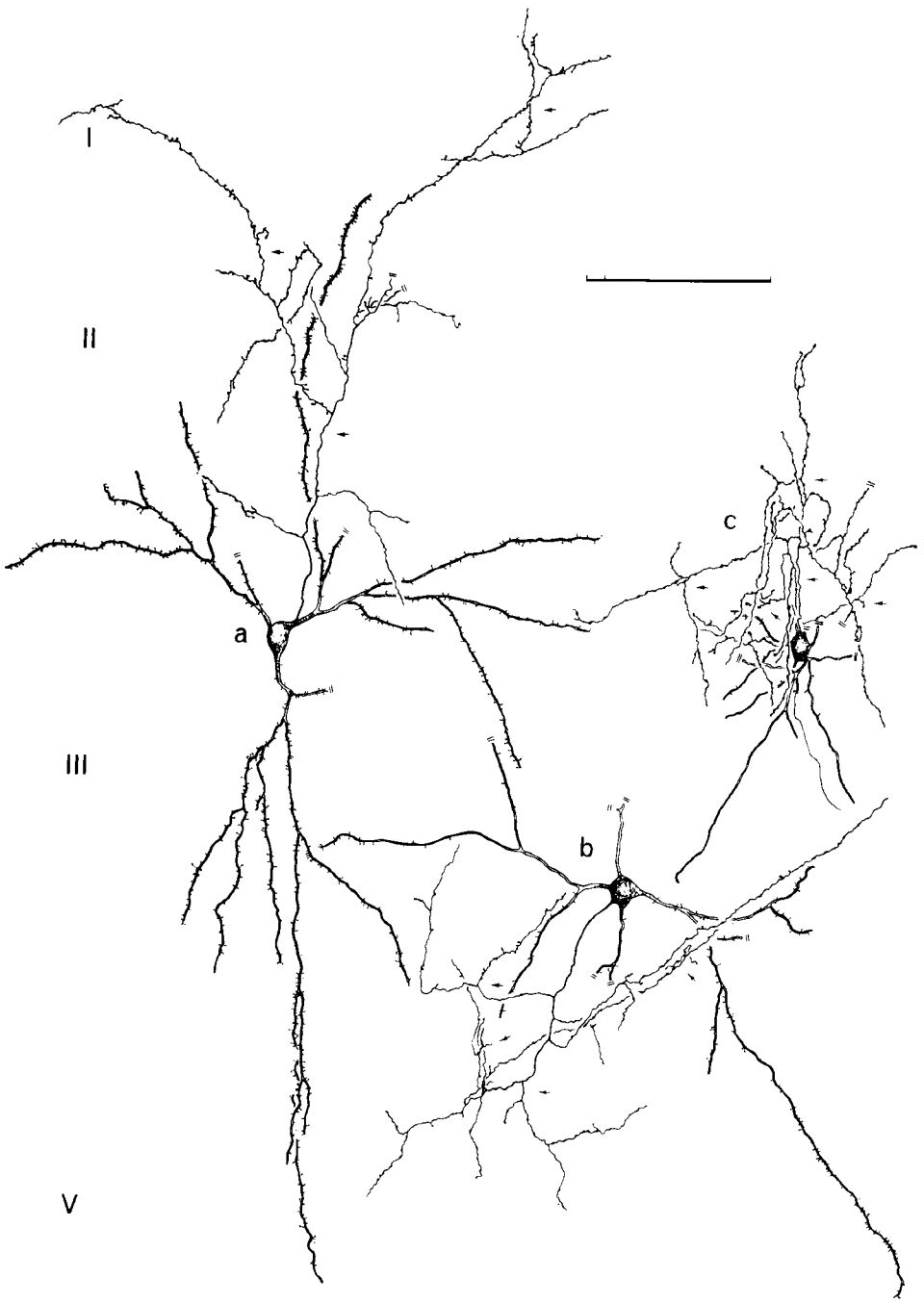

Fig. 9. Medium multipolar cells from layer III of area 24b. Cells a and b have axons which are distributed in layers III, II, and I and in layers III and V respectively. Cell c, a small or medium multipolar neuron, has an axonal plexus which is restricted to layer III. Calibration = 100 μm with a 10-μm division.

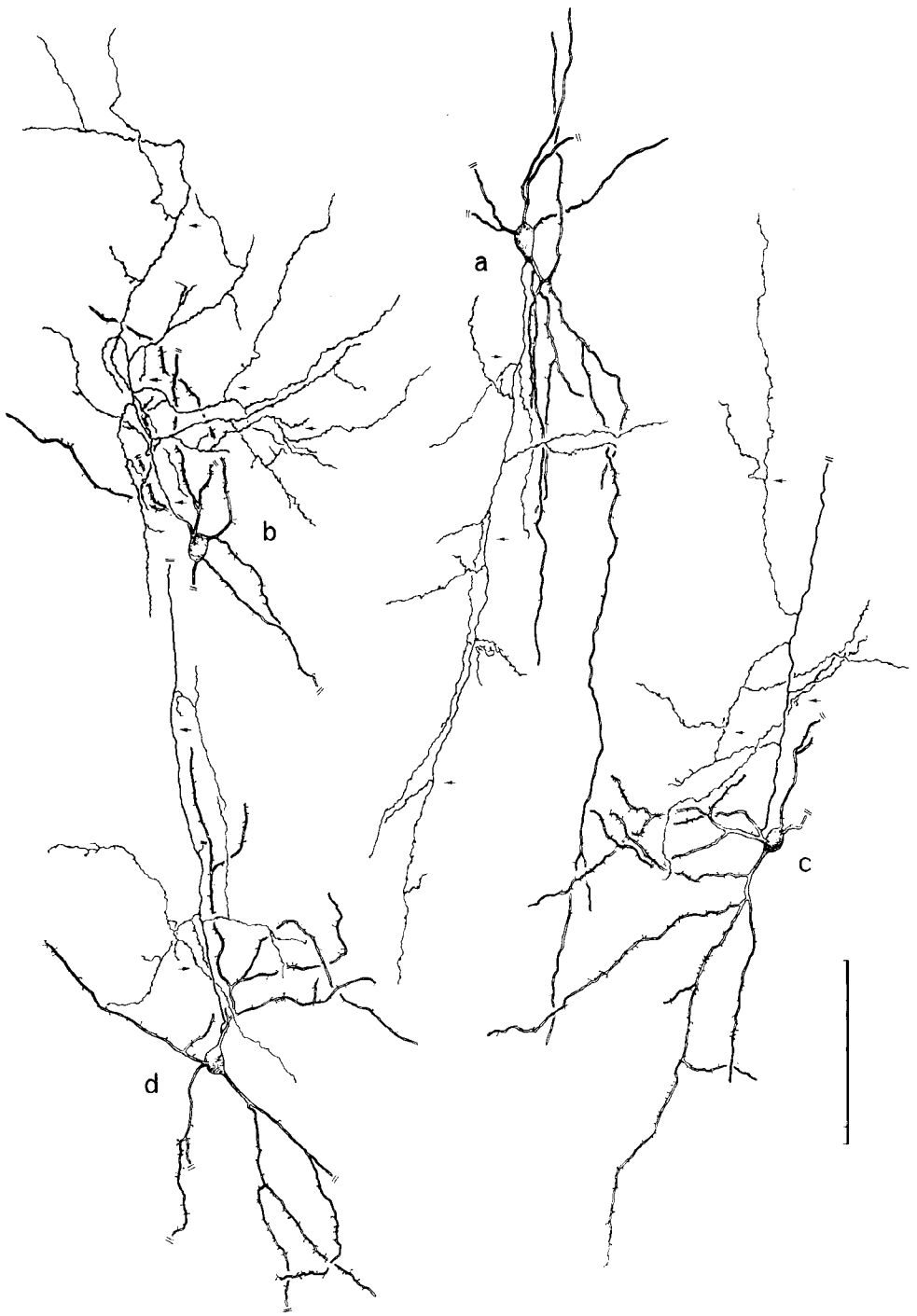

Fig. 10. Medium multipolar cells from layer V of areas 24 and 32. Calibration = 100 μm with a 10-μm division.

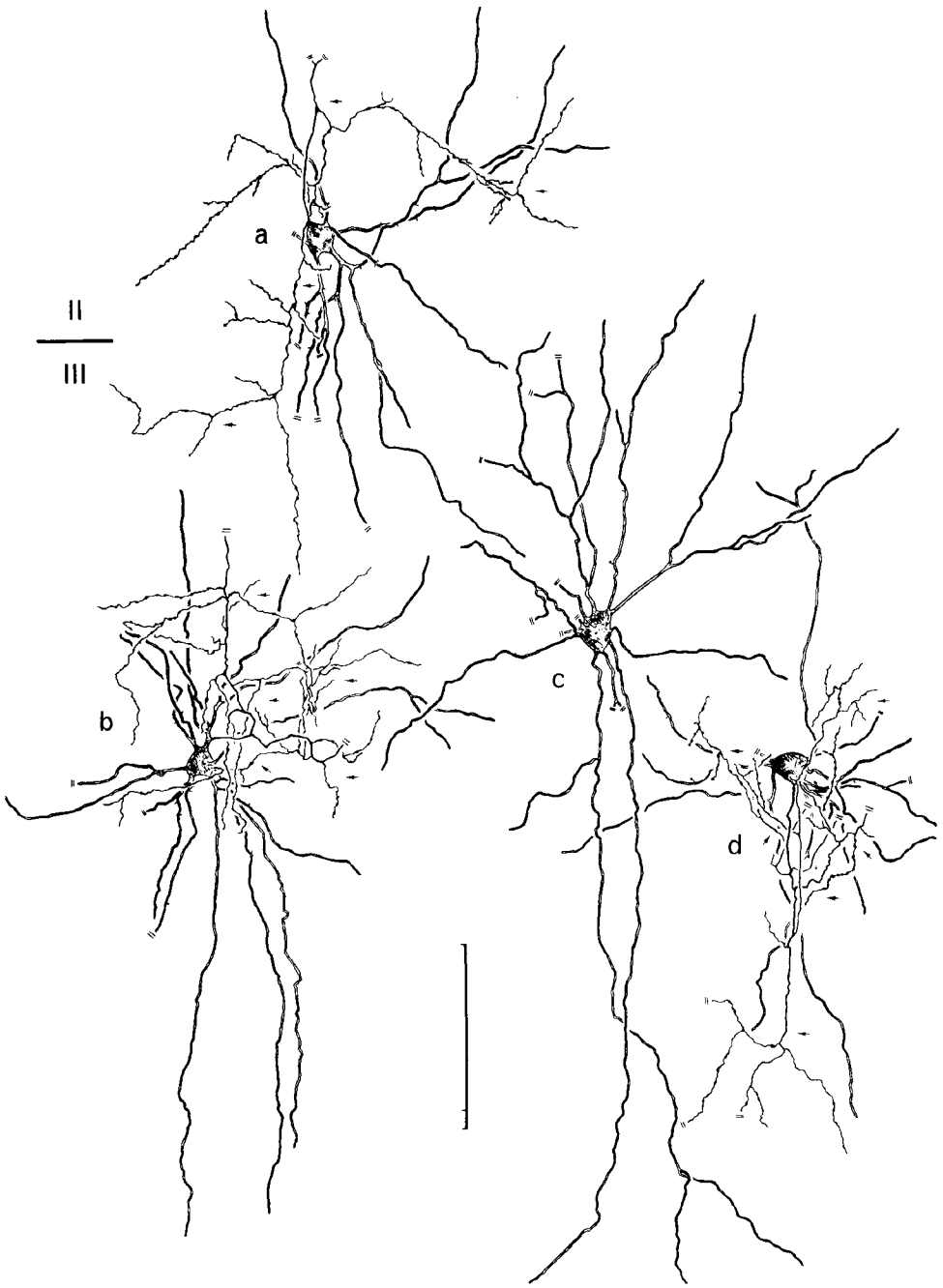

Fig. 11. Large multipolar cells in layers II–III of areas 24a and 24b. Calibration = 100μm with a 10-μm division.

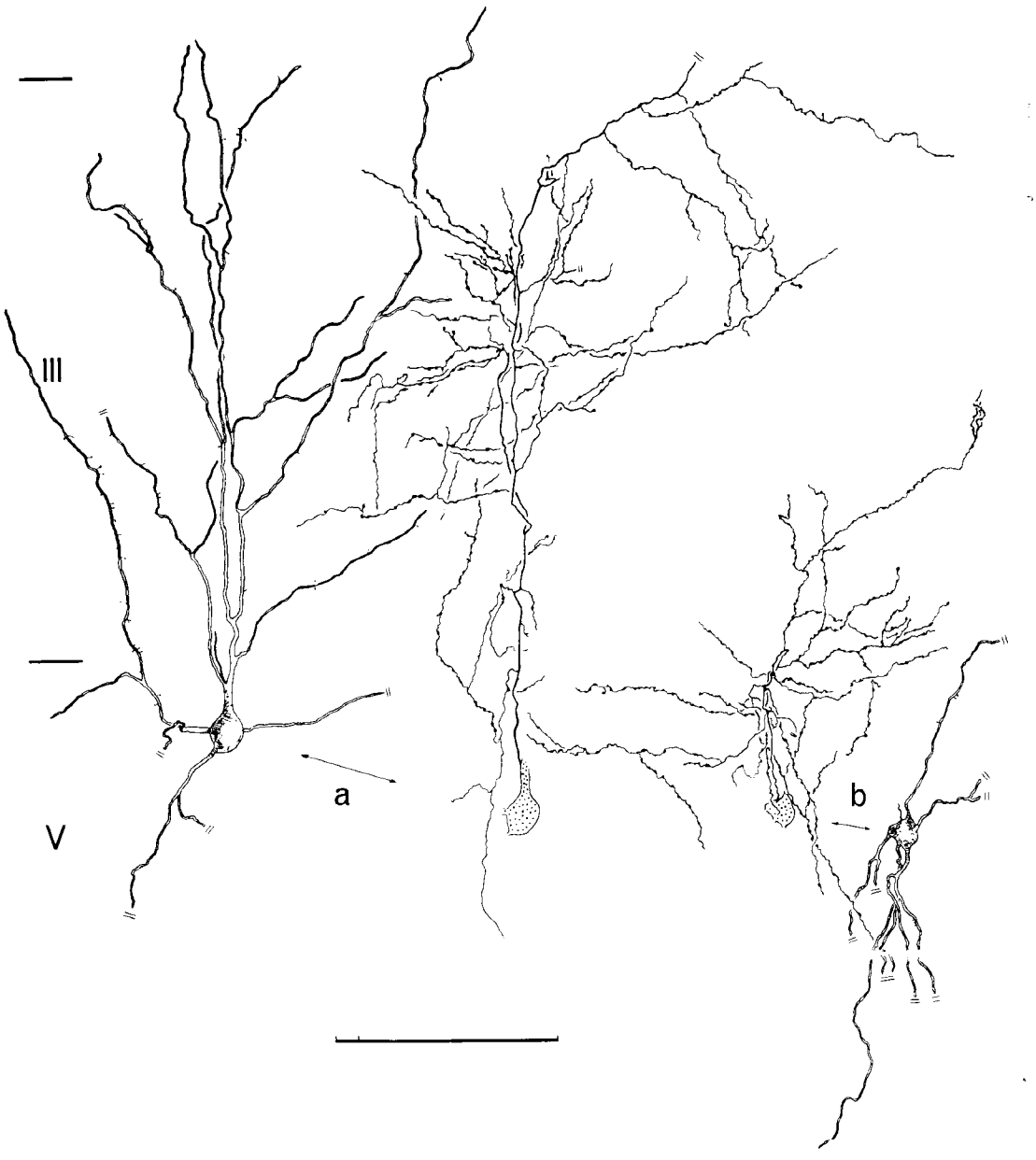

Fig. 12. Large (a) and medium (b) multipolar cells from layer V of areas 24b and 24a respectively. The dendritic and axonal arbors are presented separately. Calibration = 100 μm with a 10-μm division.

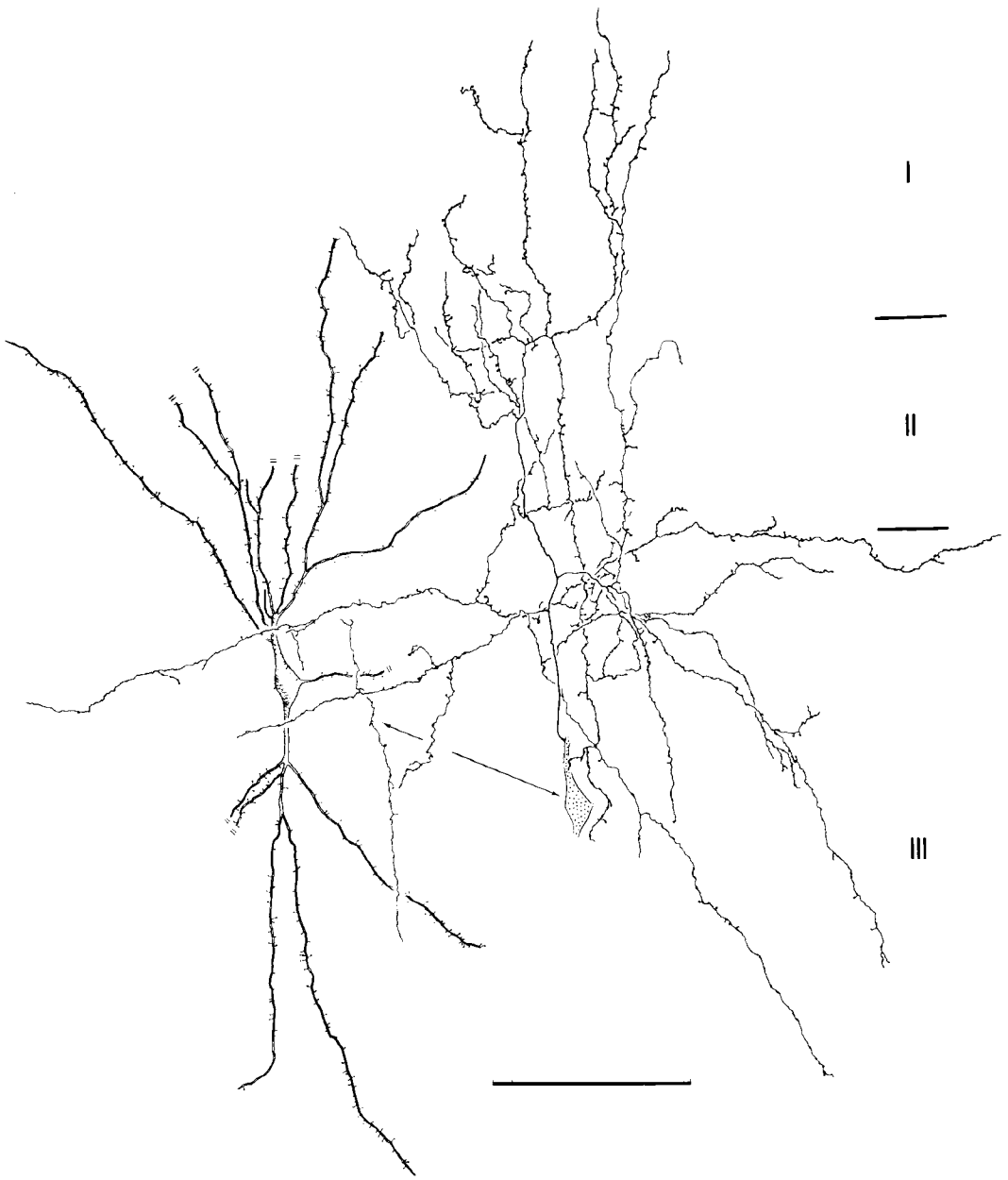

Fig. 13. Bitufted cell from layer III of area 32 with dendritic and axonal arbors drawn separately. Calibration = 100 μm with a 10-μm division.

which have a predominatly vertical orientation in layer I. The result is an axonal arbor with both a broad horizontal and vertical projection field (Fig. 14).

A second variety of bitufted cell has a "chandelier" axon. A "chandelier cell" has been described by a number of authors (e.g., Somogyi, '77; Lund et al. '79). In both cingulate and neocortical areas the axons of such bitufted cells descend from the base of the soma and produce collaterals from which extend many vertical branches, each composed of a series of large varicosities. Although the cell body of origin could not always be determined, such axonal endings are present in layer II and the top of layer III throughout areas 32 and 24 and form a very dense, intracortical plexus, which may have its terminals around the initial axon segments of pyramidal neurons, as they do in neocortex (Somogyi, '77; Peters, '80).

Bipolar cells. Bipolar cells have small, fusiform somata (8–10-μm wide) found mainly in layers III and V. A primary dendrite usually projects from both the upper and lower poles of the soma, although a third laterally extending dendrite is often encountered. These primary dendrites project some distance from the soma before branching and form a dendritic tree condensed into a long, thin, vertical field which traverses three or more layers (Fig. 15). The dendrites of such cells are, at most, only lightly spinous. The axons of these neurons arise from either the soma or proximal dendrites, and follow the form of the dendritic tree rather closely. Thus, when the

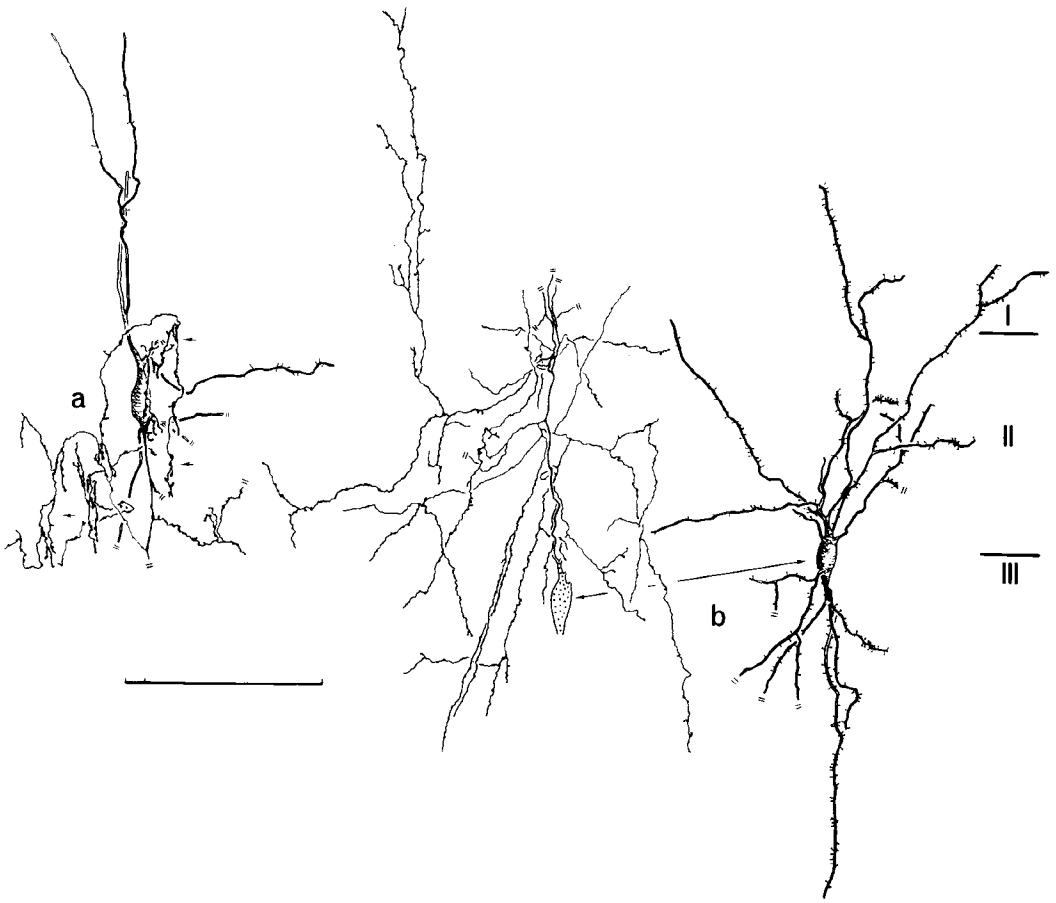

Fig. 14. Bitufted cells from area 24a and 24b respectively. Cell a has a "chandelier" axon, while cell b, whose axonal and dendritic arbors are shown separately, has an axon which projects into layer I with long horizontal branches. Calibration = 100 μm with a 10-μm division.

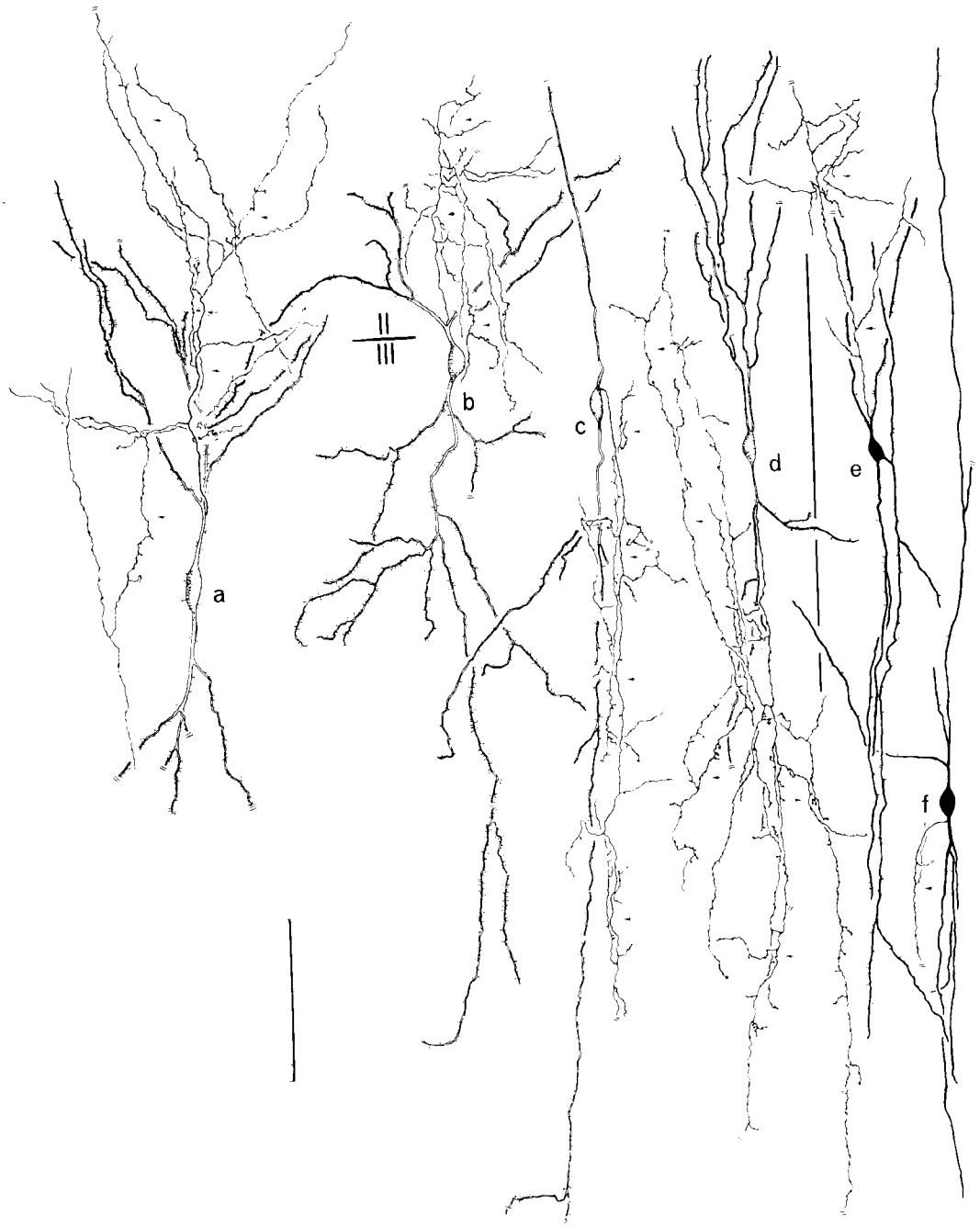

Fig. 15. Bitufted cells from layer III of area 24b (cell a) and area 32 (cell b). Bipolar cells from layer III of area 24b (cell c) and area 24a (cell d) and from layer V of area 24b (cell e) and area 29c (cell f). Arrows indicate axons, calibration = 100 μm with a 10-μm division.

axon is relatively completely impregnated and preserved (Fig. 15, c, d), it can be seen to have a restricted horizontal spread and to form many vertical branches which either ascend or descend for long distances.

In conclusion, the form of the axonal arbor of a nonpyramidal cell frequently mimics the extent and shape of the dendritic tree. Thus, small multipolar cells with limited, spherical dendritic trees may have axons which arch sharply and emit short, terminal branches. In contrast, medium and large multipolar cells have more extensive dendritic and axonal arbors which traverse two, three, or more layers. Of the fusiform cells, bitufted ones with their "hourglass" dendritic figure have extensive vertical and horizontally oriented axonal branches, while bipolar cells have narrow, vertically oriented dendritic and axonal arbors.

Stellate cells in layers II-IV of area 29. In retrosplenial granular cortex (areas 29a, b, c) stellate cells (multipolar, bitufted, and bipolar) are similar to those of areas 32, 24, and 29d with some variation due to the presence of the granular layers II–IV (Fig. 5). The following differences are recognizable:

a) Small multipolar cells are present in layer I of area 29c but most of the cytoarchitectural granularity of layers II–IV is due to fusiform and small pyramidal cells which have extrinsic instead of primarily intracortical axonal trajectories.

b) Medium multipolar cells with ascending axons are found frequently in layers, IV, V, and VI, but usually not in layers II–III.

c) Two varieties of large multipolar cells are present. Those in layers V and VI (Fig. 5, q, r) are similar to the large multipolar cells observed in anterior cortex. In addition, layer II–III contains the "large stellate" cell of Cajal ('22). A number of features distinguish this cell type (Fig. 5, l) from the large multipolar stellate cell described above (Fig. 11): The dendrites are heavily spinous and the axon has a large diameter which descends in the cortex, emits three to five ascending collaterals, and according to Cajal, enters the cingulum bundle. This neuron is similar to the long projecting star cells in layer II of entorhinal cortex described by Cajal ('11) and Lorente de Nó ('33).

d) Bitufted and bipolar cells have not been seen in layers II–IV, but they do appear in layer V.

DISCUSSION

Cytoarchitecture

In experimental anatomical studies Kreig's (46a,b) cytoarchitectural map of the rat cerebral cortex has been the one most frequently used for parcellation of the various areas. However, Kreig's analysis of the medial cingulate surface shows a number of departures from Brodmann's ('09) original description. First, area 32 is restricted to a small region immediately surrounding the rostrum of the corpus callosum—a region which is part of Brodmann's area 24. Area 32 actually extends much further rostrally (see also Krettek and Price, '77). Second, Kreig divided area 29 into granular areas 29b and c, both of which are located dorsal to the corpus callosum and apparently included Brodmann's ventral areas 29a and b in the presubiculum (area 27). These two divisions of area 29 should not be considered part of the presubiculum, since as can be readily ascertained, neurons in the granular layer of area 27 are less densely packed and more evenly distributed than in retrosplenial cortex. Area 27 also lacks the internal plexiform layer found in area 29b (unpublished observations).

Over the years Brodmann's ('09) area 29e has proven to be a most enigmatic area to investigators. Cortex represented as area 29e by Brodmann has been treated in the following ways. First, it has been included as part of the presubiculum or retrosplenial fields (Kreig, '46a,b; Caviness, '75). Second, although some investigators recognize a distinct cortical area in this region, they consider it to be part of the parahippocampal cortex and call it the postsubiculum (Rose and Woolsey, '48; Swanson et al., '78). Finally, Blackstad ('56) has identified a different area as 29e, but this area 29e is apparently not continuous with retrosplenial cortex, from which it differs greatly in terms of its cytoarchitecture, and does not correspond topographically with area 29e of Brodmann. Indeed, it is interposed between the parasubiculum and presubiculum. These difficulties might be resolved by making Blackstad's area 29e a division of the presubiculum (area 27b?) and continuing to apply the term postsubiculum for Brodmann's area 29e, as has been done in the present analysis.

Finally, although reasons have already been presented for discontinuing the use of Brodmann's area 23 in the rat, it is particularly important from a comparative point of view to

consider this issue further. In the monkey area 23 occupies a substantial portion of the posterior cingulate cortex between agranular retrosplenial and parietal cortex (Vogt, '76). Cytoarchitecturally area 23 in the monkey is granular isocortex, for it has a distinctly granular layer IV. If an extrapolation can be made to other species, area 23 should be a truly granular neocortex that occurs between retrosplenial agranular cortex (area 29d) and more lateral neocortex. Brodmann's ('09) area 23 in the rat is actually a transitional region between areas 24 and 29, where the granularity is forming in layers II–III, and not in layer IV. Thus, compared to the well-developed area 23 of the monkey, Brodmann's area 23 in the rat is located in a different place topographically and lacks a granular layer IV. Furthermore, the neocortex lateral to area 29d does not satisfy the criteria for area 23. In this context area 23 is a component of the cingulate cortex of higher mammals but not that of rodents.

Cellular morphology

The drawings of cells in cingulate cortex made by Cajal ('22) are not among his best renditions. Because many of these drawings were made from very young animals, it has been difficult to compare the form of neurons in cingulate cortex with those in other cortical areas in the adult rat. In light of the present findings, the following comparisons can now be made among neurons in cingulate and other cortical areas. First, the fusiform pyramid is the only neuron which is truly unique to cingulate cortex. Second, the large stellate cell is similar to the star cell of entorhinal cortex, but such large, spiny neurons are not found in neocortex. Third, all other nonpyramidal or stellate cells in cingulate cortex have counterparts in neocortex.

The unique form of the fusiform pyramid is associated with the unique cytoarchitecture of retrosplenial granular cortex (areas 29a-c). The oval somata of these neurons are located in layer II–III, where cell packing density is similar to that of the dentate gyrus. However, in contrast to the dentate gyrus granule cell (Cajal, '11), the fusiform pyramid posesses a tapered apical dendrite that branches to form an apical tuft in layer I and only one basally projecting dendrite, which descends to arborize in the less cell-dense layer IV. The form of the apical dendrite, the moderate to heavy number of dendritic spines, and presence of a long projecting axon with three to six ascending collaterals indicates that this cell is a variety of small pyramid.

The large stellate cell has a round somata, many dendrites with spines, and a large-caliber, efferent axon (Cajal, '22). These characteristics are similar to those described for the star cell of entorhinal cortex (Cajal, '11; Lorente de Nó, '33)—a cell which is known to project into the hippocampus (Steward and Scoville, '76). However, there are not as many large stellate cells in cingulate cortex as there are star cells in entorhinal cortex in which these neurons form a distinct layer II. Also, it seems to be the case that the large stellate cells of cingulate cortex often extend more dendrites into layer I than they do into lower layers, resulting in a more asymmetric dendritic tree than is formed by the star cell.

Besides the presence of fusiform pyramids and large stellate cells, all other pyramidal and stellate cells so far encountered in cingulate cortex have exact counterparts in neocortex. These include small, medium, and large stellate (multipolar) cells (Valverde, '71), bitufted and bipolar cells (Feldman and Peters, '78), and the "chandelier" cell (Somogyi, '77; Lund et al., '79). The main difference between cingulate and neocortex seems to be in the proportion of these cells which are present, since neocortex has a more pronounced layer IV, in which stellate cells are concentrated.

This data about neuronal morphology can be used to interpret recent findings about receptor characteristics of neurons in cingulate cortex alluded to at the beginning of this article. For example, Hunt and Schmidt ('78) found that [^{125}I]-αbungarotoxin binds to receptors in layer I of cingulate cortex in the rat. However, only neurons with somata in layer II–III appear to endocytose the ligand, resulting in a number of heavily labelled somata in this layer. These cells appear to be the largest ones present in layer II–III, and since they have round somata, they are probably the large stellate cells with extrinsically projecting axons. Furthermore, Sar et al. ('78) have described the presence of enkephalinergic processes in cingulate cortex. Based on Nissl-stained preparations, these authors state that the cell bodies of the neurons surrounded by these immunoreactive processes are those of pyramidal cells. However, cingulate cortex also contains large multipolar cells which often have somata shaped similar to those of pyramidal cells (see also Feldman and Peters,

'78), so that a more thorough analysis of the dendritic and axonal arbors of the cells involved with these enkephalinergic processes must be made before it can be accepted that they are pyramidal cells. Finally, Lorén et al ('79) found that in the rat, the cingulate and perirhinal cortices have the greatest number of vasoactive intestinal polypeptite (VIP)-positive neurons. Immunoreactive VIP is present in the somata of neurons located in layers II–V and the dendritic trees of these cells are frequently similar in form to the bipolar cell, as suggested by Lorén et al. ('79). Besides bipolar cells, it is possible that some of the immunoreactive VIP neurons in layer II–III of posterior cingulate cortex are fusiform pyramidal cells, since these neurons also have oval somata and one dendrite arising from each pole.

In conclusion, the cytoarchitecture and distribution of cell types described in the present analysis provide a basis for the interpretation of future physiological and pharmacological studies of specific neurons in rat cingulate cortex. It is also reasonable to expect that the afferent and efferent connections of cingulate cortex can now begin to be analyzed in terms of these variations in cellular morphology, instead of being confined to general descriptions of which laminae the connections arise or terminate. To achieve this end, however, resort will have to be made to recently developed techniques, such as those using combined horseradish peroxidase-Golgi impregnation methods (Somogyi et al., '79) and combined degeneration-Golgi impregnation methods (Peters et al., '77).

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LITERATURE CITED

Azmitia, E.C. and M. Segal (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J. Comp. Neurol. *179:*641–668.

Brodmann, K. (1909) Vergleichende Lokalisations Lehre de Grosshirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues. Leipzig: J.A. Barth, pp. 324.

Cajal, S. Ramón y (1911) Histologie du Systeme Nerveux de l'homme et des Vertébrés. Tome II. Paris: Maloine.

Cajal, S. Ramón y (1922) Estudios sobre la Fina Estructura de la Corteza Regional de los Roedores. I. Corteza suboccipital (Retrosplenial de Brodmann). Trab.Lab. biol. Univ. Madrid *20:*1–30.

Caviness, V.S., Jr. (1975) Architectonic map of neocortex of the normal mouse. J. Comp. Neurol. *164:*247–264.

Emson, P.C., and G.F. Koob (1978) The origin and distribution of dopamine-containing afferents to the rat frontal cortex. Brain Res. *142:*249–267.

Feldman, M.L., and A. Peters (1978) The forms of non-pyramidal neurons in the visual cortex of the rat. J. Comp. Neurol. *179:* 761–794.

Hunt, S., and J. Schmidt (1978) Some observations on the binding patterns of αbungarotoxin in the central nervous system of the rat. Brain Res. *157:*213–232.

Jones, B.E., A.E. Halaris, M. McIlhany, and R.Y. Moore (1977) Ascending projections of the locus coeruleus in the rat. I. Axonal transport in central noradrenalin neurons. Brain Res. *127:*1–21.

Kreig, W.J.S. (1946a) Connections of the cerebral cortex I. The albino rat. A. Topography of the cortical areas. J. Comp. Neurol. *84:*221–275.

Kreig, W.J.S. (1946b) Connections of the cerebral cortex. I. The albino rat. B. Structure of the cortical areas. J. Comp. Neurol. *84:*277–323.

Krettek, J.E., and J.L. Price (1977) The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat. J. Comp. Neurol. *171:*157–192.

Lewis, M.S., M.E. Molliver, J.H. Morrison, and H.G.W. Lidov (1979) Complementarity of dopaminergic and noradrenergic innervation in anterior cingulate cortex of the rat. Brain Res. *164:*328–333.

Lidov, H.G.W., R. Grazanna, and M.E. Molliver (1980) The serotonin innervation of the cerebral cortex in the rat—an immunohistochemical analysis. Neurosciences *5:*207–227.

Lindvall, O., A. Björklund, R.Y. Moore, and U.Stenevi (1974) Mesencephalic dopamine neurons projecting to neocortex. Brain Res. *81:*325–331.

Lorén, I., P.C. Emson, J. Fahrenkrug, A. Björklund, J. Alumets, R. Håkanson, and F. Sundler (1979) Distribution of vasoactive intestinal polypeptide in the rat and mouse brain. Neurosciences *4:*1953–1976.

Lorente de Nó, R. (1922) La corteza Cerebral del Ratón (Primera contribucion—La cortexa acústica.). Trab. Lab. biol. Univ. Madrid *20:*41–104.

Lorente de Nó, R. (1933) Studies on the structure of the cerebral cortex. I. The area entorhinalis. J. Psych. U. Neurol. *45:*381–438.

Lund, J.S., G.H. Henry, C.L. MacQueen, and A.R. Harvey (1979) Anatomical organization of the primary visual cortex (area 17) of the cat. A comparison with area 17 of the Macaque monkey. J. Comp. Neurol. *184:*599–618.

Marin-Padilla, M. (1967) Number and distribution of the apical dendritic spines of the layer V pyramidal cells in man. J. Comp. Neurol. *131:*475–490.

Palkovits, M., J.M. Saavedra, D.M. Jacobowitz, J.S. Kizer, L. Záborszky, and M.J. Brownstein (1977) Serotonergic innervation of the forebrain: Effect of lesions on serotonin and tryptophan hydroxylase levels. Brain Res. *130:*121–134.

Peters, A. (1970) The fixation of central nervous tissue and the analysis of electron micrographs of the neuropil with special reference to the cerebral cortex. In W.J.H. Nauta and S.O.E. Ebbesson (eds.): Contemporary Research Methods in Neuroanatomy. New York: Springer-Verlag, pp. 56–76.

Peters, A. (1980) Neuronal organization in rat visual cortex. In J.R. Harrison and R.L. Holmes (eds.): Progress in Anatomy. Cambridge: Cambridge University Press, (in press).

Peters, A., E.L. White, and A. Fairén (1977) Synapses between identified neuronal elements. An electron microscopic demonstration of degenerating axon terminals synapsing with Golgi impregnated neurons. Neurosci. Letters *6:*171–175.

Rose, M. (1912) Histologische Lokalisation der Grosshirndrinde bei kleinen Saügetieren (Rodentia Insectivora, Chiroptera). J.F. Psychol. U. Neurol. *19:*389–479.

Rose, J.E., and C.N. Woolsey (1948) Structure and relations

of limbic cortex and anterior thalamic nulcei in rabbit and cat. J. Comp. Neurol. *89:*279–340.

Sar, M., W.E. Stumpf, R.J. Miller, K.-J. Chang, and P. Cuatrecasas (1978) Immunohistochemical localization of enkephalin in rat brain and spinal cord. J. Comp. Neurol. *182:*17–38.

Schierhorn, H., K. Doedens, and I. Nagel (1972) Über die laminäre Zuordnung der apikalen Dendritenspines der Lamina-V-Pyramidenzellen in der sensomotorischen Hirnrinde der Albinoratte. Gegenbaurs morph. Jahrb. *118:*465–487.

Smith, W.K. (1945) The functional significance of the rostral cingular cortex as revealed by its responses to electrical excitation. J. Neurophysiol. *8:*241–255.

Somogyi, P. (1977) A specific axo-axonal interneuron in the visual cortex of the rat. Brain Res. *136:*345–350.

Somogyi, P., A.J. Hodgson, and A.D. Smith (1979) An approach to tracing neuron networks in the cerebral cortex and basal ganglia. Combination of Golgi staining, retrograde transport of horseradish peroxidase and anterograde degeneration of synaptic boutons in the same material. Neurosciences *4:*1805–1852.

Steward, O., and S.A. Scoville (1976) Cells of origin of entorhinal cortical afferents to the hippocampus and fascia dentata of the rat. J. Comp. Neurol. *169:*347–370.

Swanson, L.W., and B.K. Hartman (1975) The central adrenergic system. An immunofluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine-β-hydroxylase as a marker. J. Comp. Neurol. *163:*467–506.

Swanson, L.W., J.M. Wyss, and W.M. Cowan (1978) An autoradiographic study of the organization of intrahippocampal association pathways in the rat. J. Comp. Neurol. *181:*681–716.

Valverde, F. (1965) Studies on the Piriform Lobe. Cambridge: Harvard University Press, pp. 4–69.

Valverde, F. (1970) The Golgi method. A Tool for Comparative Structural Analyses. In W.J.H. Nauta and S.O.E. Ebbesson (eds.): Contemporary Research Methods in Neuroanatomy. New York: Springer-Verlag, pp. 12–31.

Valverde, F. (1971) Short axon neuronal subsystems in the visual cortex of the monkey. Intern. J. Neurosci. *1:*181–197.

Vogt, B.A. (1974) A reduced silver stain for normal axons in the central nervous system. Physiol. Behav. *13:*837–840.

Vogt, B.A. (1976) Retrosplenial cortex in the rhesus monkey: A cytoarchitectonic and Golgi study. J. Comp. Neurol. *169:*63–98.

Walker, A.E. (1940) A cytoarchitectural study of the prefrontal area of the Macaque monkey. J. Comp. Neurol. *73:*59–86.